

University of Groningen

## Respiratory Responses to Stimulation of Branchial Vagus Nerve Ganglia of a Teleost Fish

Ballintijn, C.M.; Luiten, P.G.M.

*Published in:*  
Respiration Physiology

*DOI:*  
[10.1016/0034-5687\(83\)90044-0](https://doi.org/10.1016/0034-5687(83)90044-0)

**IMPORTANT NOTE:** You are advised to consult the publisher's version (publisher's PDF) if you wish to cite from it. Please check the document version below.

*Document Version*  
Publisher's PDF, also known as Version of record

*Publication date:*  
1983

[Link to publication in University of Groningen/UMCG research database](#)

*Citation for published version (APA):*

Ballintijn, C. M., & Luiten, P. G. M. (1983). Respiratory Responses to Stimulation of Branchial Vagus Nerve Ganglia of a Teleost Fish. *Respiration Physiology*, 51(2), 241-257. [https://doi.org/10.1016/0034-5687\(83\)90044-0](https://doi.org/10.1016/0034-5687(83)90044-0)

**Copyright**

Other than for strictly personal use, it is not permitted to download or to forward/distribute the text or part of it without the consent of the author(s) and/or copyright holder(s), unless the work is under an open content license (like Creative Commons).

The publication may also be distributed here under the terms of Article 25fa of the Dutch Copyright Act, indicated by the "Taverne" license. More information can be found on the University of Groningen website: <https://www.rug.nl/library/open-access/self-archiving-pure/taverne-amendment>.

**Take-down policy**

If you believe that this document breaches copyright please contact us providing details, and we will remove access to the work immediately and investigate your claim.

*Downloaded from the University of Groningen/UMCG research database (Pure): <http://www.rug.nl/research/portal>. For technical reasons the number of authors shown on this cover page is limited to 10 maximum.*

## RESPIRATORY RESPONSES TO STIMULATION OF BRANCHIAL VAGUS NERVE GANGLIA OF A TELEOST FISH

C. M. BALLINTIJN, B. L. ROBERTS\* and P. G. M. LUITEN

*Zoölogisch Laboratorium der Rijksuniversiteit, Kerklaan 30, 9751 NN Haren, The Netherlands*

**Abstract.** The effects of electrical stimulation of epibranchial vagus ganglia upon respiration of the carp were investigated. Single shocks evoked fast twitch responses in a number of respiratory muscles with latencies around 18 msec to the beginning and 30–35 msec to the peak of activity.

Shocks given during abduction decreased the respiratory cycle duration by shortening abduction and accelerating adduction. Stimuli given throughout most of adduction also shortened the respiratory cycle, accelerating the adduction only. These responses are similar to vagally mediated lung receptor reflexes of mammals.

Stimulation with short trains of pulses produced a rapid expansion–contraction movement. This movement resembles in all respects (shape, time in the respiratory cycle, muscle coordination) the intermediate expansion of a normal coughing movement.

Continual stimulation at frequencies close to the normal respiratory rate had a synchronising influence upon respiration, speeding up or slowing down its rate.

HRP applied to the third vagal ganglion showed that there is a small projection of this ganglion to the nucleus intermedius facialis, although the majority of sensory fibres terminate in the vagal lobe. The nucleus intermedius facialis is already known to connect directly with the respiratory motor centres and thus might provide a pathway for the fast twitch response. A projection was also found to the nucleus ambiguus; in mammals this nucleus plays an important role in the regulation of respiratory movements.

Breathing pattern	Respiratory muscles
Carp	Vagus nerve
Respiratory centres	Water breather

It can be concluded that the pattern of respiratory movements of fishes is produced in the hindbrain because transections at the caudal and rostral limits of the rhombencephalon do not abolish respiration (Shelton, 1959). Microelectrode recordings from the hindbrain of paralyzed fish reveal the presence of respiratory-

*Accepted for publication 2 November 1982*

\* On leave from the laboratory of the Marine Biological Association, Plymouth, U.K.

patterned neurons that retain their rhythmicity in the absence of any rhythmic sensory input (Ballintijn, 1972; Ballintijn and Bamford, 1975; Ballintijn and Roberts, 1976).

The basic respiratory pattern of the mammal is also generated by central oscillators. The output of these oscillators is profoundly influenced by the activity of stretch receptors in the lungs, supplied by the vagus nerve (d'Angelo, 1978; Cohen, 1969, 1974; Cohen and Feldman, 1977; Trippenbach and Milic-Emili, 1977), as can be mimicked by vagal stimulation (Feldman and Gautier, 1976; Iscoe *et al.*, 1979; Trenchard, 1977). These effects are the basis of the fact that rhythmic respiration continues after the central generators have been lesioned, if the vagi are intact (Feldman and Gautier, 1976; St. John *et al.*, 1972; Hugelin, 1980).

The gills of fishes are well provided with mechanoreceptors (Sutterlin and Saunders, 1969) supplied by vagal rami (except for the first gill arch which receives a glossopharyngeal innervation). The function of this vagal input to the central nervous system is unknown although a respiration-retarding effect of vagal stimulation has been reported for an elasmobranch fish (Satchell, 1959).

The present research was undertaken to investigate the central connections of the branchial branches of the vagus nerve in a teleost fish and to examine the effects of stimulation of these branches on respiration. Some of the results have been reported in brief elsewhere (Ballintijn and Roberts, 1981).

## Material and methods

The physiological experiments were performed on 13 carp, *Cyprinus carpio*, (17–25 cm long) maintained under light MS-222 (tricaine methane sulfonate) anaesthesia. Each fish was almost completely immersed in a tank of water, the head rigidly held by clamps on the orbital ridges and the body supported in a plastic-foam trough. The anaesthetic dose was initially 75 mg/L of water and was lowered to about 60 mg/L when the fish was fixed prior to surgery. After surgery the concentration was decreased to about 40 mg/L and then left unchanged throughout the experiment.

The first three epibranchial ganglia of the vagus nerve were exposed by a dorsal approach. A hole was cut in the pterotic bone with a circular dental saw and the tissues outside the brain case overlying the ganglia were removed. Although some muscle tissue (*e.g.*, a substantial part of the adductor operculi) had to be removed, bleeding was seldom troublesome and could be controlled by cotton-wool swabs. Because of its size and easy access, the third vagal ganglion was used for stimulation in most cases.

Stimuli were applied with bipolar stainless-steel electrodes inserted into the ganglia (which were 1–1.5 mm wide). The pointed, uninsulated tips of the electrodes were 150–200  $\mu\text{m}$  in diameter and were placed about 500  $\mu\text{m}$  apart.

The stimuli consisted of pulses (0.5 msec duration; 0.5–4 mA) given singly or in

short trains (5–8 pulses per train, 20 msec interval) via a stimulus isolator (Neurolog, Devices Ltd.). The stimuli were presented free-running at adjustable frequency (0.25–100 Hz for single pulses and 0.25–20 Hz for trains) or respiration-triggered with an adjustable phase relation to the respiratory cycle.

The respiratory movements were recorded with a mechano-electrical transducer in the following way. A light-weight, pivoted arm rested against the anterior half of the operculum, and moved a vane between a LED and a phototransistor, converting the movement into an electrical signal. Because the movement was recorded from the anterior part of the operculum it represents a combination of both opercular and hyomandibular motion and therefore is a fair estimate of the total respiratory volume changes of the combined buccal and opercular cavities (Ballintijn, 1969).

For cycle-triggered stimulation a pulse was derived from the movement record at maximum adduction. This pulse triggered the stimulus generator via an adjustable delay circuit to give a single pulse, or a short train, at a predetermined phase of the respiratory cycle.

Electromyograms of important respiratory muscles (adductor mandibulae, levator hyomandibulae and dilator operculi) were recorded with 50  $\mu$ m varnish-insulated copper wires, bared at the tip for about 0.5 mm, and amplified with Grass P15 preamplifiers (filter setting: low 30–100 Hz; high 3 kHz).

Because the adductor operculi and one or two of the epibranchial muscles on the operated side were severely damaged during surgery, opercular movement and electromyograms were mostly recorded contralaterally. However, comparison with the ipsilateral movements and myograms revealed no significant differences between the responses of the two sides.

Anatomical studies were performed in five additional animals where the third vagal ganglion was exposed as described above. Horseradish peroxidase was delivered into the ganglion either by pressure injection or by electrophoresis. For the former, 20  $\mu$ l of a 40% solution (Sigma type VI in teleost saline) was injected into the ganglion in several penetrations. For iontophoresis a current of 2  $\mu$ A was applied for 15 min to glass micropipettes filled with a 10% HRP solution in 0.01 M HCl. Following the injection, the dorsal hole was closed with dental cement and the animals allowed to survive for 10–12 days at 18 °C. The animals were then reanaesthetized with an overdose of MS-222 and perfused through the heart initially with 30 ml teleost saline and then with 100 ml 1% paraformaldehyde – 1.5% glutaraldehyde – 4% sucrose in 0.05 M phosphate buffer at pH = 7.4. After fixation the brains were embedded in 20% gelatin and post-fixed en bloc overnight.

The brains were cut at 40  $\mu$ m on a freezing microtome and every second section was reacted for HRP according to the benzidine–hydrochloride procedure of De Olmos and Heimer (1977) and counterstained with neutral red. Both anterogradely filled afferent and retrogradely labelled efferent neuron somata and fibres were reconstructed in a series of transverse drawings (fig. 6).

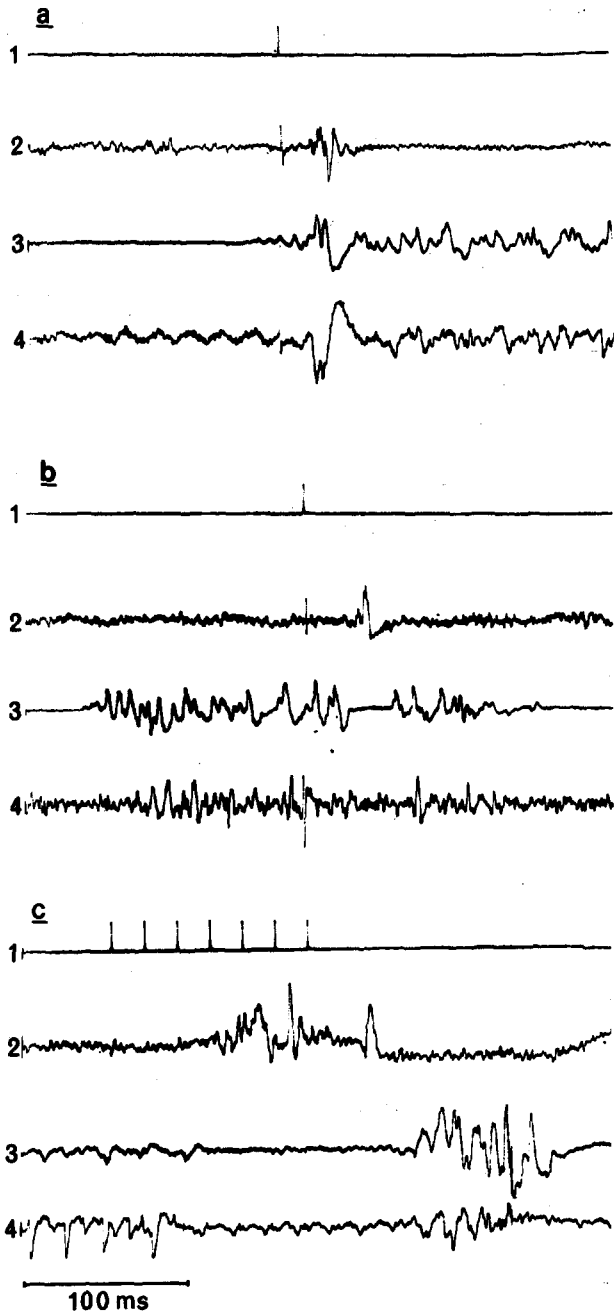


Fig. 1. The direct muscle twitch in three respiratory pump muscles in response to electrical stimulation of a branchial vagus ganglion. (a) A single stimulus at the start of levator hyomandibulae and dilator operculi contraction elicits an activation in all three muscles after about 18 msec with a peak at 30-35 msec. (b) A single stimulus near the end of levator hyomandibulae and dilator operculi contraction elicits an activation in the adductor mandibulae coincident with a suppression of activity in the other two muscles. (c) A train of pulses elicits a longer activation in the adductor mandibulae coincident with inactivation, followed by activation in the levator hyomandibulae and dilator operculi. 1 = stimulus marker; 2 = EMG adductor mandibulae; 3 = EMG levator hyomandibulae; 4 = EMG dilator operculi. Stimuli: a: 0.5 msec, 1.6 mA; b: 0.5 msec, 1.6 mA; c: 0.5 msec, 50 Hz; 1 mA.

## Results

Under the conditions of these experiments, with continuous light anaesthesia, the carp respired regularly (around 1 Hz) and made coughs at intervals of a few normal respirations. Each respiration consisted of an abduction and an adduction phase (fig. 2), resulting in alternating expansion and contraction of the respiratory cavities. The abduction and adduction muscles did not show overlap in activity during normal respiration. Some overlap did occur, however, during the cough when the mouth remained closed while the abduction muscles were active (pattern fully described by Ballintijn, 1969).

The breathing pattern was modified by stimulation of the vagal ganglia (ganglia 1–3), which evoked responses in the respiratory muscles. The responses of both ipsi- and contralateral muscles were similar whichever ganglion was stimulated and were unchanged if the nerve was cut distal to the stimulation site. The responses were not dependent on the position of the stimulating electrode in the ganglion.

### DIRECT TWITCH RESPONSES OF THE MUSCLES

Figure 1a shows recordings from three respiratory muscles when a single stimulus was applied to the contralateral vagus ganglion. At all phases of the respiratory cycle the adductor mandibulae showed an evoked response which consisted of one to a few potentials with a latency of about 18 msec to the beginning and 30–35 msec to the peak of the reaction. The reaction of the levator hyomandibulae and the dilator operculi was more complex and depended on the timing of the stimulus in relation to the respiratory cycle. When these muscles were inactive, or at the start of their normal respiratory activity, a response commenced after about 18 msec, as in the adductor mandibulae (fig. 1a). However, stimulation during the later part of their normal contraction resulted in a period of suppression of the ongoing activity, concurrent with the response in the adductor mandibulae (fig. 1b). These responses were more evident in some fish than in others and probably depended on the state of the preparation and on the depth of anaesthesia. When present, the response was always more pronounced and more constant in the adductor mandibulae than in the other two muscles. In some fish no responses to single shocks were ever seen, even though responses to small trains of stimuli were readily obtained.

With low frequency stimulation (*ca* 0.2–1 Hz instead of a single pulse) the response declined markedly and became uncertain after the first stimulus, but if the stimuli were given at rates faster than 1 Hz the muscle response declined much more slowly and with higher frequencies even increased in amplitude (fig. 4).

When small trains of stimuli (0.5 msec, 50 Hz and 7 pulses) were used the threshold was slightly lower but the responses seen in the respiratory muscles were similar to those obtained with single shocks (fig. 1c). The duration of the

responses was longer, however, and in the levator hyomandibulae and dilator operculi the period of inactivation which prevents overlap between abduction and adduction activity was more evident because no activity occurred during the adductor mandibulae reaction. The period of suppressed activity in the levator hyomandibulae and dilator operculi was followed by a short burst of action potentials.

The largest response was always obtained to the first stimulus train of a series but, in contrast to single shock stimulation, a limited response with increased latency persisted to subsequent stimulus presentations.

#### THE EFFECT OF VAGUS STIMULATION ON RESPIRATORY MOVEMENTS

Vagal stimulation, apart from evoking a direct muscle twitch response, had a general effect on the respiratory movements, depending on the time during the respiratory cycle when the stimulus was presented. In fig. 2 are displayed mechano-transducer records of the respiratory movements and the concomitant electromyograms of the adductor mandibulae (A) and the levator hyomandibulae (L) when single pulse stimuli (0.5 msec; 4 mA) were applied progressively later during the respiratory cycle. When the stimulus was given during the abduction phase (ab, fig. 2: 1,2,3) the records show that the abduction was terminated prematurely with an abrupt cessation of levator hyomandibulae activity. The latency of this reaction decreased for stimuli given later during abduction. Following the cessation of levator hyomandibulae activity, a strong activation occurred in the adductor mandibulae, after which its normal activity terminated earlier (fig. 2: 1, 2, 3). The result was a shortening of the plateau section (p) of the adduction phase (fig. 2: 1, 2, 3).

A stimulus given during the adduction phase itself produced a temporary increase in adductor mandibulae activity only. For stimuli given early during adduction this resulted in a marked decrease in adduction duration, again by reduction of the plateau (fig. 2: 4, 5, 6). Stimuli presented towards the end of adduction no longer shortened its duration but left it unchanged or slightly prolonged (fig. 2: 7).

Because vagal stimulation generally shortened the ongoing cycle by terminating the abduction and/or reducing adduction time, subsequent respiratory cycles, although themselves unchanged in shape, were shifted forward in phase with respect to the cycles that preceded stimulation.

The effect of stimulation with small trains of pulses (0.5 msec; 0.7 mA; 5 pulses at 20 msec interval) during abduction was similar to that of single shock stimulation: levator hyomandibulae activity stopped, abduction was terminated and adduction shortened (fig. 3:1). However, in addition, a rapid expansion-contraction movement of the respiratory cavities was elicited (fig. 3: 1, 2, 3). This movement was clearly locked to the stimulus, as it was evoked irrespective of the phase relation between stimulus and respiratory cycle. Its maximum abducted amplitude was constant and independent of stimulus timing. It appeared to be evoked by extremely high

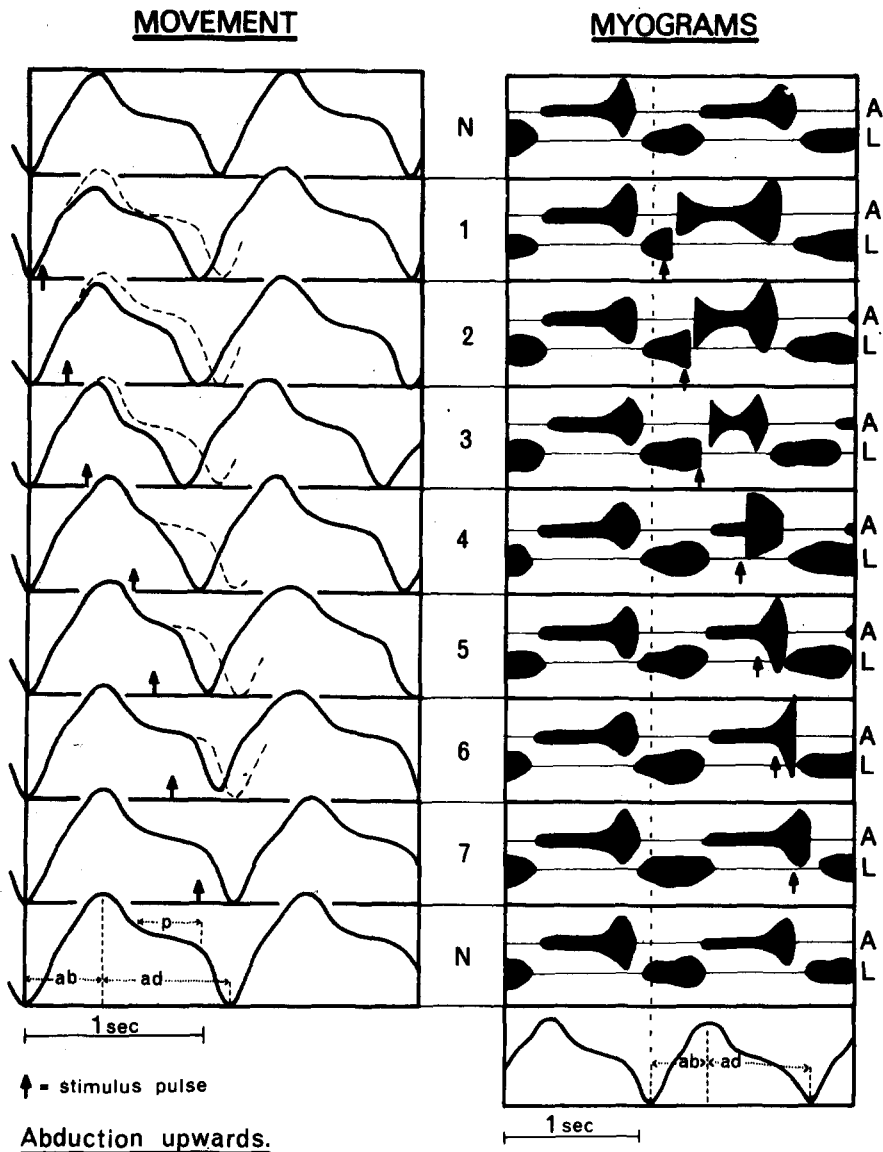


Fig. 2. The effect on respiratory movement (left) and electromyograms (right) of electrical stimulation of a branchial vagus ganglion. The stimuli are presented progressively later during the respiratory cycle. In the movement graphs the normal movement without stimulation is drawn in a broken line. Stimuli delivered during abduction (1-3) terminate levator hyomandibulae activity and cause an increase in adductor mandibulae activity amplitude together with a decrease in its duration. The movement of the abduction phase is terminated and the adduction shortened. Stimuli presented during adduction (4-7) increase adductor mandibulae activity amplitude and decrease its duration. The adduction phase is shortened but the effects decrease, the later the stimulus is given. N = normal, unstimulated. 1-7 = stimuli presented progressively later during the respiratory cycle. ↑ = stimulus (0.5 ms, 4 mA). A = adductor mandibulae; L = levator hyomandibulae; ab = abduction half cycle (expansion); ad = adduction half cycle (contraction); p = plateau.



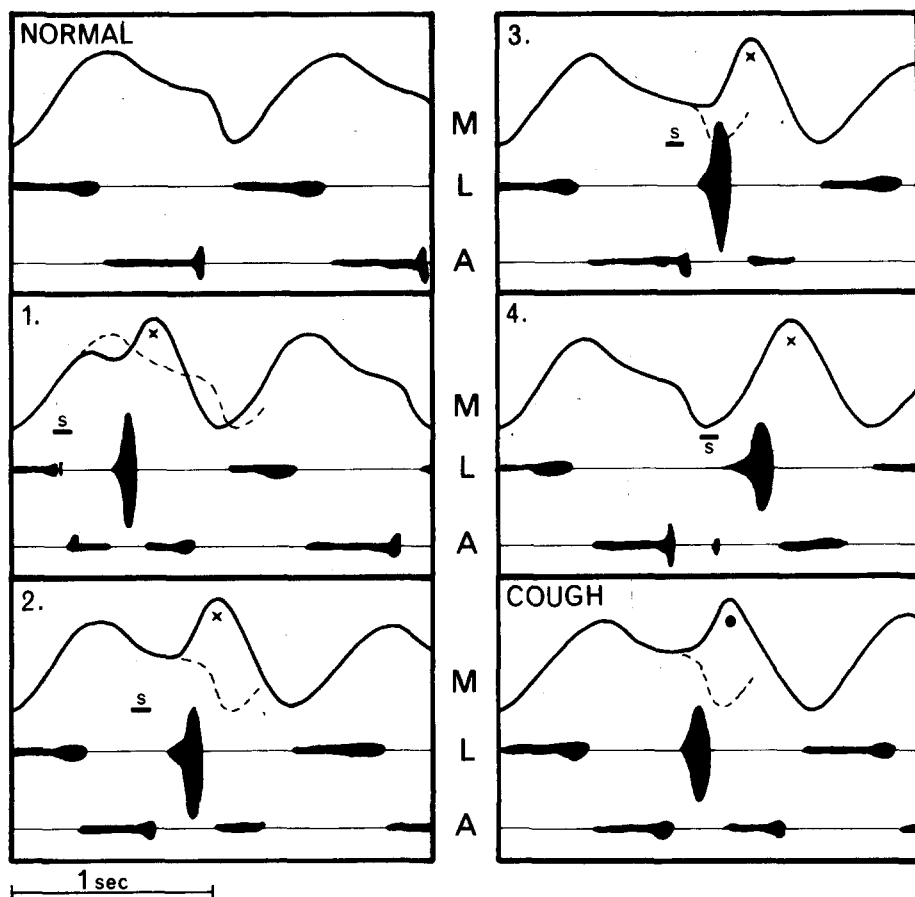


Fig. 3. The effects of electrical stimulation with trains of pulses on the respiratory movement and the electromyograms of the levator hyomandibulae and the adductor mandibulae. The normal, unstimulated movement is drawn in a broken line. The stimuli are delivered progressively later during the respiratory cycle (1-4). They result, apart from the phase modifying effect shown in fig. 2 in a rapid expansion-contraction movement ( $\times$ ) which is related to an extremely powerful levator hyomandibulae contraction followed by an adductor mandibulae contraction. The expansion-contraction movement ( $\times$ ) only occurs during the adduction phase, even for stimuli delivered during abduction (1) and bears strong resemblance to the intermediate expansion ( $\bullet$ ) of the cough (compare 2 with cough). 1-3 = stimuli presented progressively later during the respiratory cycle. s = stimulus burst (5 pulses; 50 Hz; 0.5 msec; 0.7 mA); M = movement; L = levator hyomandibulae; A = adductor mandibulae;  $\times$  = expansion-contraction movement;  $\bullet$  = intermediate expansion of cough.

intensity bursts of activity in the levator hyomandibulae (fig. 3) and the dilator operculi. Because of an initial gradual build-up the latency between stimulus and onset of muscle activity could not be determined exactly. It depended markedly on the phase relation between stimulus and respiration for stimuli given during the abduction phase but was nearly constant for stimuli presented during adduction. The values were about 350 msec for stimuli at the start of abduction (not illustrated),

300 msec for stimuli halfway through abduction (fig. 3: 1) and 160–170 msec for all stimuli presented between early-adduction and end-adduction (fig. 3: 2, 3 and 4).

The time interval between the stimulus and the maximum electromyogram activity or the maximum amplitude of the movement could be measured more accurately than the latency. The intervals were respectively: 420 and 560 msec, 375 and 500 msec (fig. 3: 1), 315 and 430 msec (fig. 3: 2), 300 and 430 msec (fig. 3: 3) and finally 320 and 430 msec (fig. 3: 4). The changes in latency for stimuli delivered during abduction result from the fact that the expansion–contraction movement is never executed during the abduction phase of respiration but only during the adduction phase, or is interpolated between two respiratory cycles, as occurs in response to a stimulus given at the end of adduction (fig. 3: 4).

Comparision between the expansion–contraction movement elicited by vagal stimulation and the intermediate expansion during a normal cough (description of cough: Ballintijn, 1969) reveals the exact similarity between the two, both with respect to movement and muscle activity patterns (compare fig. 3: 2 with fig. 3: cough).

As the level of anaesthesia was increased the expansion–contraction response and the respiratory cycle shortening effects failed together, suggesting that these processes have similar thresholds. Nevertheless, they appear to be separable for the following reasons:

(a) The respiratory cycle shortening effects produced by single shocks were equivalent to those resulting from train stimulation but in the former the expansion–contraction response was absent. (In rare cases a slight hump, suggesting an expansion could be detected).

(b) The cycle shortening effects persisted during prolonged stimulation once every respiratory cycle while the expansion–contraction response adapted fairly quickly and could no longer be detected, even in the electromyograms.

#### THE EFFECT OF STIMULATION OF THE VAGUS ON THE RESPIRATORY RHYTHM

Electrical stimulation of a vagus ganglion at frequencies of approximately the same range as the respiratory rhythm (*i.e.*, around 1 Hz) modified the frequency of the respiratory cycle (fig. 4a). Stimulation at higher frequencies (around 10 Hz) enhanced the general level of abduction and increased the respiratory frequency (fig. 4b). At still higher stimulus frequencies (fig. 4c, 20 Hz) the respiratory cycles, which were very short at the start of the stimulus, gradually became longer and eventually ceased in tonic abduction (fig. 4c). At very high stimulus frequencies (about 100 Hz) respiratory movements sometimes broke through this maintained abduction (fig. 4d).

The direct muscle twitch response was very pronounced in the electromyograms especially at higher stimulus frequencies (up to several tens of Hz, figs. 3b and c).

Figure 5 shows the effect of different stimulus frequencies close to the respiration

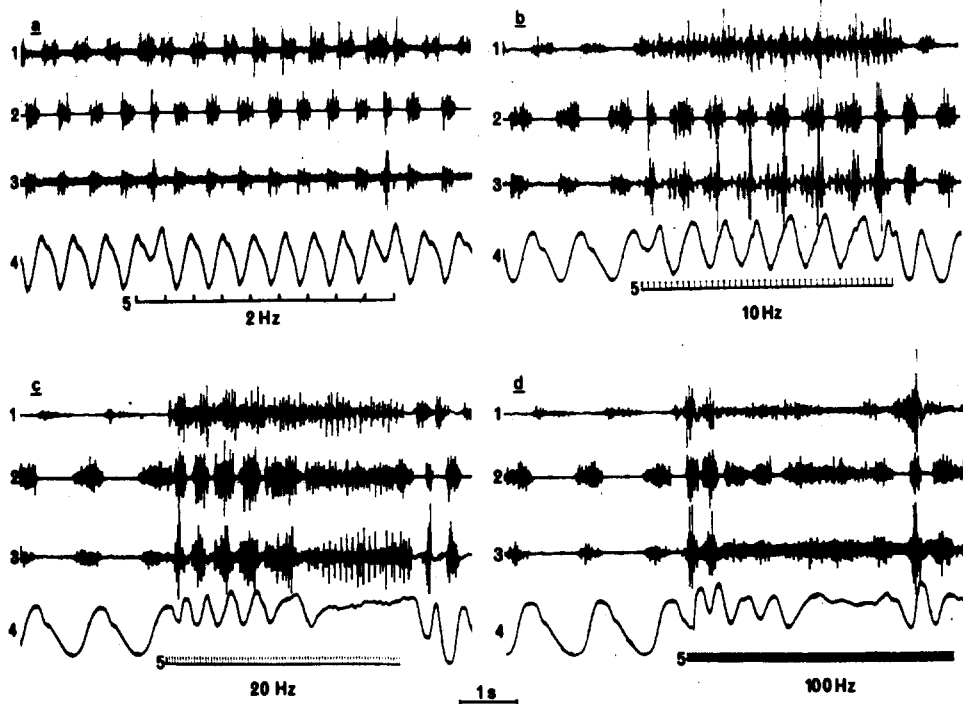


Fig. 4. The effect of stimulation of a vagus nerve at different frequencies on respiration. The duration of the respiratory cycle decreases and the general level of abduction increases markedly with increasing stimulus frequencies (a and b). At higher frequencies respiration eventually stops at maximum abduction (c) but may finally break through during very high frequency stimulation (d). Note the frequency modulation of the electromyograms by the direct response which is especially clear at 10 Hz and still present at 20 Hz and also the fact that the adductor mandibulae with higher stimulus frequencies tends to develop a tonic firing pattern. Stimuli 0.5 msec, 1.6 mA. 1 = EMG adductor mandibulae; 2 = EMG levator hyomandibulae; 3 = EMG dilator operculi; 4 = movement operculum (abduction upwards); 5 = duration of stimulus train.

frequency on a fish respiring steadily with a cycle period of slightly less than 700 msec. Stimulation at intervals greater than 1000 msec produced no change in the respiratory pattern, except to reduce slightly the tendency to cough (not illustrated). At 1000–800 msec stimulus interval the stimulus tended to shift progressively the respiratory period towards the stimulus period (fig. 5a). At around 700 msec stimulus interval no coughs occurred and the respiratory frequency was closely linked with the stimulus frequency (fig. 5b).

As the stimulus interval was shortened, synchrony became less precise and the respiratory periods tended to shift to multiples of the stimulus frequency. Thus with 500 msec stimulation, the respiratory periods, initially at 500 msec, shifted gradually to 1000 msec; at 400 msec the respiration period became 800 msec (fig. 5c), while at 300 msec the final period was 900 msec. These latter values are of course longer than the spontaneous base frequency (700 msec).

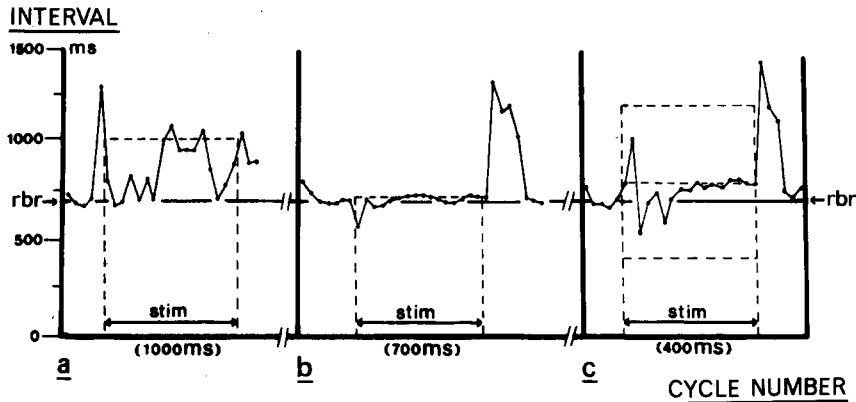


Fig. 5. The effect on the respiratory period of electrical stimulation of a vagus ganglion with periods of 1000, 700 and 400 msec. In the first case (a) respiration shows a tendency to synchronize with the stimulus. In the second (b) the respiratory period is stabilised by the stimulus and the coughs, which occur normally after every few respiratory cycles, are completely suppressed and in the third (c) respiration tends to lock to about double the stimulus interval. Stimuli 0.5 msec, 2.4 mA. rbr = respiratory base rhythm. The vertical broken lines indicate beginning and end of stimulus periods, the horizontal broken lines the stimulus interval.

The effects of continual stimulation on respiratory cycle duration varied considerably from fish to fish. This probably reflects the fact that continual stimulation disturbs the base frequency which is set by factors other than the stimulated vagus ganglion alone. Nevertheless, the general pattern illustrated in fig. 5 could be detected in these fish.

Continual stimulation with small trains instead of single shocks produced the same change in respiratory frequency although the disturbing effect was greater, partly as a result of the interpolation of the expansion-contraction movement, and the respiratory pattern usually became more erratic.

#### CENTRAL CONNECTIONS OF THE THIRD VAGAL GANGLION

Horseradish peroxidase applied to the third vagal ganglion resulted in labelling of both the sensory afferent and the motor efferent neurons by anterograde and retrograde transport respectively. The sensory fibres split into a major superficial and a smaller deeper sensory root immediately after entering the brain (fig. 6). Both sensory roots surround the well-developed sensory vagal lobe (L10) and give off small fascicles of labelled fibres that penetrate the cellular core of the lobe. Here the individual sensory fibres terminate in a complicated branching pattern.

Apart from this major projection to the sensory vagal lobe, two smaller additional projections were observed. A number of fibres that run via the deep sensory root leave the main bundle (fig. 6b), pass medially and were seen to terminate in an area of spindle-shaped cells called the nucleus intermedius facialis

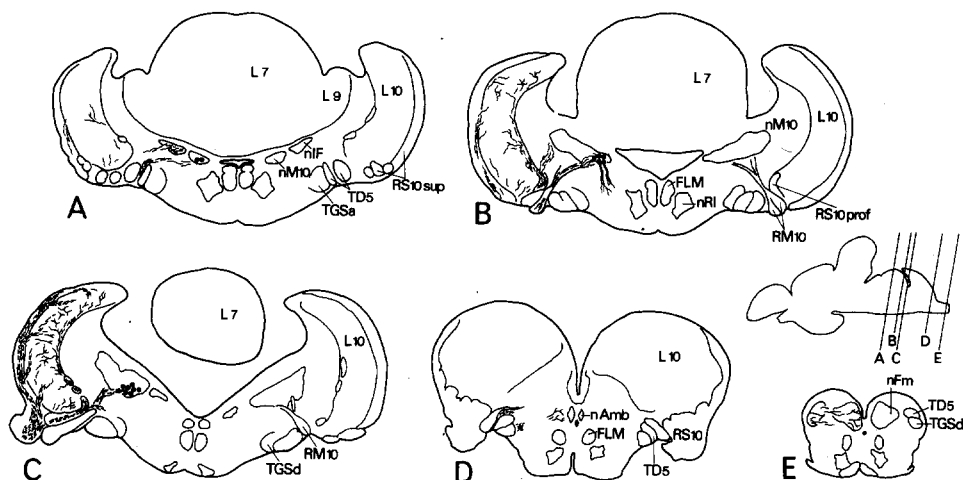


Fig. 6. Series of transverse sections from rostral (a) to caudal (e) showing the course and termination of HRP labelled fibres following injections in the third vagal ganglion on the left side. FLM = fasciculus longitudinalis medialis; L7 = sensory facial lobe; L9 = sensory glossopharyngeal lobe; L10 = sensory vagal lobe; nAmb = nucleus ambiguus; nFm = nucleus funicularis medialis; nIF = nucleus intermedius facialis; nM10 = vagal motor nucleus; nRI = nucleus reticularis inferior; RM10 = motor vagal root; RS10 prof = deep sensory vagal root; RS10 sup = superficial sensory vagal root; TD5 = descending trigeminal tract; TGSa = ascending secondary gustatory tract; TGSd = descending secondary gustatory tract.

(NIF, fig. 6a). A second group of sensory fibres split from the deep sensory root slightly more caudally. Here they pass over the motor root of the vagal nerve and take a caudalwards turn. They can be followed adjacent to the descending trigeminal and descending secondary gustatory tracts into the first cervical segments of the spinal cord where they reach their target cells in the medial funicular nucleus (nFm, fig. 6e). More rostrally, some of these fibres terminate close to the nucleus ambiguus (nAmb, fig. 6d).

The motor and sensory roots of the vagus are joined at the ganglionic level, as is the case with trigeminal and facial nerves in teleosts. Proximal to the ganglion, however, the motor roots are separate from the sensory root and leave the brain as an isolated bundle lateroventrad to the sensory bundles. The motor neurons that became labelled after third vagal ganglion injections take a rather rostral position and constitute a well-defined cluster in the medial aspects of the huge vagal motor nucleus. It could be observed that several of these motor neurons bear long dendrites that branch out into the ill-defined grey (adjacent lateral) of the reticular formation (fig. 6b).

## Discussion

The physiological experiments show that stimulation of the vagus nerve has a marked effect on the respiratory movements of the carp. Stimulation of just one of the relevant six vagus ganglia with single pulses produced a short latency activation of several respiratory muscles – called here the muscle twitch response – and a change in length of the respiratory phase. When short trains of stimuli were used a stereotyped expansion–contraction movement was elicited as well.

### MUSCLE TWITCH RESPONSE

The muscle twitch response involved a short latency reaction of all three muscles studied which declined markedly with repeated low-frequency stimulation. At higher stimulus frequencies the response was facilitated and followed frequencies of several tens of Hz. As the response was still obtained after the vagus had been sectioned distal to the stimulation site, a central action must be involved and peripheral effects that would follow stimulus-evoked movements of the gill arches are inessential.

The twitch response's short latency (18 msec) and its following of high-frequency stimulation indicate that it is acting fairly directly on the motor areas (V for adductor mandibulae; VII for levator hyomandibulae and dilator operculi) rather than on the respiratory centres.

The latency of 18 msec is composed of an afferent, central and efferent transmission component. The efferent component for the adductor mandibulae and the levator hyomandibulae is nearly 5 msec in carp of the size used (Ballintijn and Alink, 1977). Experiments on sensory transmission in maxillary branches of the trigeminal nerve suggest that this is markedly slower than motor transmission and can be estimated at about 7–10 msec. This leaves 3–5 msec for the central delay. The total latency of 18 msec of the vagus reaction studied in the present paper is in the same range as that of the proprioceptive load compensation reflex of the adductor mandibulae (around 15–20 msec, preliminary report, Ballintijn and Schuitmaker, 1980) which, with neuroanatomical tracer methods, was shown to be bisynaptic (Luiten, 1979). It is probable, therefore, that the present vagally elicited twitch response also involves a bisynaptic neural circuit. This conclusion receives support from our HRP studies which reveal a sensory vagus projection to the intermediate facial nucleus which was shown in earlier studies (Luiten and van der Pers, 1977) to have direct connections with the trigeminal and facial motor nuclei innervating the respiratory muscles. The fact that during low-frequency repetitive stimulation the response rapidly adapts and with high-frequency stimulation is facilitated indicates that the vagal input also affects the overall sensitivity of the circuit, possibly via higher elements of the respiratory centre.

If we assume that the stimulus mimicked, even if crudely, strong stimulation

of gill receptors this response could be part of a defence reaction that protects the delicate gills.

#### THE RAPID EXPANSION-CONTRACTION

The rapid expansion-contraction movement resulting from short train stimulation clearly exhibits the characteristics of a gill cleaning reflex. In fact its movement pattern and muscle coordination closely resemble the normal gill cleaning movement (the 'cough', Ballintijn, 1969). It is noteworthy that the latencies for stimuli presented during abduction, the phase of respiration when coughs never occur, are prolonged. As a result, the expansion-contraction reaction is postponed to the adduction phase, just as in a normal cough.

#### MODIFICATION OF THE RESPIRATORY PATTERN

Unlike the direct twitch response, the modification of the respiratory pattern brought about by vagal stimulation depended completely on the time during the cycle when the stimulus was presented and did not decline with continual stimulation. Only the ongoing cycle was modified and subsequent cycles were unchanged. The cycle was always shortened when stimuli were given during the abduction phase because abduction was terminated and adduction was increased in speed. Stimulation during adduction only accelerated adduction. These results suggest that vagal information contributes to the switching of the respiratory phases.

#### SYNCHRONISING THE RESPIRATORY CYCLE TO THE STIMULUS

The stimulation of only one vagus ganglion shifted the respiratory frequency to a limited extent. The respiratory rate tended to synchronise in a one to one ratio only with stimuli given at frequencies close to the natural frequency and faster stimuli synchronized with multiples of the stimulus interval. These results show that vagal input is a factor in determining the timing of respiration.

The neuroanatomical findings provide the first experimental evidence on the major sensory projections to the vagal lobes. They confirm Herrick's (1906a, b, 1907) classical description of sensory lobe organization in fish. Moreover, some hitherto unknown projections have been found. The most interesting in relation to the electrophysiological results is the projection to the intermediate facial nucleus (nIF, fig. 6a) because, as is mentioned above, it seems to be part of the neural circuit responsible for the direct response. In connection with the control of respiration the projection to the nucleus ambiguus (nAmb, fig. 6d) is also of interest. In mammals this nucleus, together with the nucleus retroambiguus, con-

stitutes the ventral bulbar respiratory nucleus which, as with the dorsal bulbar respiratory nucleus, receives vagal information from lung stretch receptors influencing respiratory phase switching and rhythm generation. On comparative grounds, therefore, the effects of branchial vagus nerve stimulation upon phase switching and respiratory rhythm generation in teleosts, described above, thus could well be mediated via the nucleus ambiguus, as in mammals.

#### COMPARISON WITH THE MAMMALIAN RESPIRATORY SYSTEM

Although the respiratory mechanism in fish differs in important aspects from that of the mammal, there are also some similarities and some comparisons can be made between the two systems (Ballintijn, 1982). Thus, the expansion phase (abduction) can be likened to inspiration in the mammal, which is also an expansion, and the contradiction phase (adduction) of fish respiration can be compared to expiration.

##### *Direct twitch response and rapid expansion-contraction movement*

Vagal stimulation in mammals also produces direct effects which may have the same kind of circuitry as these responses of fish, although different nerves and muscles are involved. Thus, Iscoe *et al.* (1979) found a very small fast contralateral response in the phrenic nerve (latency 6 sec) in response to low intensity vagal stimulation and, with stronger stimulation, an additional strong bilateral response after 18 msec. The latter was accompanied by a visible muscle contraction: a gasp or sigh (or inspiratory excitatory reflex to lung inflation; Cohen, 1969) like the expansion-contraction response which was found in the carp (fig. 3). In comparing the latencies it should be borne in mind that transmission in a mammal is much faster than in fish. In the cat the responses are also mediated via only a few synapses and are independent of the influence of the vagus on the phase switching mechanism.

##### *Phase switching*

It is now generally held for mammalian respiration that vagal input from lung stretch receptors during inspiration terminates this phase in a trigger-like fashion (as can be inferred from the development of the phrenic motor activity) and reduces the duration of the next expiration (d'Angelo, 1978; Cohen, 1969, 1974, 1975; Cohen and Feldman, 1977; Iscoe *et al.*, 1979; Trenchard and Milic-Emili, 1977). This is in complete agreement with our results for stimulation given during expansion in the carp (fig. 2). Vagal lung-stretch-receptor input during expiration in the mammal results in prolongation of that phase in contrast to the reduction we observed with vagal nerve stimulation given during contraction in the carp (fig. 2). This may indicate a difference in organization between the two groups. However, a reduction in expiration time is observed in the rabbit in response to vagal input from lung irritant receptors (Davies *et al.*, 1978). Alternatively, our



results may therefore indicate nociceptor stimulation. Nothing is known of nociceptors in carp but in elasmobranchs Poole and Satchell (1979) and Satchell (1978) reported the existence of nociceptors and of 'type J' receptors in the gills.

#### *Synchronising the respiratory oscillator*

Rhythmic respiration is still possible in a cat with intact vagi even though the central respiratory oscillators (pneumotaxic centre and reticular oscillator) have been inactivated (Feldman and Gautier, 1976; Hugelin, 1980; St. John *et al.*, 1972) and this indicates the importance of vagal input.

In the anaesthetized paralyzed cat the frequency of the respiratory oscillator, as indicated by efferent phrenic discharge, was found by Cohen (1969) to adjust itself to the frequency of the artificial respiration pump, when pump volume and frequency were suitably adjusted. Hugelin (1980), citing unpublished work from Benchetrit, provides further evidence of vagal entrainment of respiration in the mammal.

The carp used in the present experiments were spontaneously breathing and only lightly anaesthetised. Nevertheless, they showed a marked tendency to adjust their breathing frequency to the frequency of vagus stimulation or multiples thereof, even when direct muscle responses were absent. It seems justifiable therefore to conclude that in the carp, as in the mammal, vagal input may play an important role in determining the respiratory rhythm.

#### **Acknowledgements**

The authors appreciate the help given by Mrs. J. Poelstra-Hiddinga and Mrs. H. Lochorn-Hulsebos in the preparation of the manuscript, S. H. Leenstra (Agricultural University, Wageningen) in providing the carp; J. Holtman and G. Bakker for fish maintenance, and W. J. Beukema and his staff and J. H. Nijboer for care of the equipment.

#### **References**

- Ballintijn, C. M. (1969). Muscle coordination of the respiratory pump of the carp. *J. Exp. Biol.* 50: 569–591.
- Ballintijn, C. M. (1972). Efficiency, mechanics and motor control of fish respiration. *Respir. Physiol.* 14: 125–141.
- Ballintijn, C. M. and O. S. Bamford (1975). Proprioceptive motor control of fish respiration. *J. Exp. Biol.* 62: 99–114.
- Ballintijn, C. M. and J. L. Roberts (1976). Neural control of proprioceptive load matching in reflex respiratory movements of fishes. *Fed. Proc.* 35: 1983–1991.
- Ballintijn, C. M. and G. M. Alink (1977). Identification of respiratory motor neurons in the carp and determination of their firing characteristics and interconnections. *Brain Res.* 136: 261–276.
- Ballintijn, C. M. and J. J. Schuitmaker (1980). Proprioceptive compensation of brief load changes in teleost respiratory muscles during normal respiration. *Proc. I.U.P.S.* XIV: 812 p.

- Ballintijn, C. M. and B. L. Roberts (1981). A Hering-Breuer-type reflex from the gill arches of a teleost fish. 3rd Congr. E.S.C.P.B., Noordwijkerhout, The Netherlands, 55 p.
- Ballintijn, C. M. (1982). The control of respiration in fishes and mammals. In: *Exogenous and Endogenous Influences on Metabolic and Neural Control*, ed. by A. D. F. Addink and N. Spronk. Oxford, Pergamon Press, pp. 127-140.
- Cohen, M. I. (1969). Discharge patterns of brainstem respiratory neurons during Hering-Breuer reflex evoked by lung inflation. *J. Neurophysiol.* 32: 356-374.
- Cohen, M. I. (1974). The genesis of respiratory rhythmicity. In: *Central Rhythmic and Regulation*, ed. by W. Umbach and H. P. Koepchen. Stuttgart, Hippokrates-Verslag.
- Cohen, M. I. (1975). Phrenic and recurrent laryngeal discharge patterns and the Hering-Breuer reflex. *Am. J. Physiol.* 228: 1489-1496.
- Cohen, M. I. and J. L. Feldman (1977). Models of respiratory phase switching. *Fed. Proc.* 36: 2367-2374.
- D'Angelo, E. (1978). Central and direct vagal dependent control of expiratory duration in anaesthetised rabbits. *Respir. Physiol.* 34: 103-119.
- Davis, A., M. Dixon, D. Callana, A. Huszczuk, J. G. Widdicombe and J. C. M. Wise (1978). Lung reflexes in rabbits during pulmonary stretch receptor block by sulphur dioxide. *Respir. Physiol.* 34: 83-101.
- De Olmos, J. and L. Heimer (1977). Mapping of collateral projections with the HRP-method. *Neurosci. Lett.* 6: 107-113.
- Feldman, J. L. and H. Gautier (1976). Interaction of pulmonary afferents and pneumotactic centre in control of respiration patterns in cats. *J. Neurophysiol.* 39: 31-44.
- Herrick, C. J. (1906a). The central gustatory paths in the brain of bony fishes. *J. Comp. Neurol.* 15: 385-456.
- Herrick, C. J. (1906b). On the centers for taste and touch in the medulla oblongata of fishes. *J. Comp. Neurol.* 16: 403-456.
- Herrick, C. J. (1907). A study of the vagal lobes and funicular nuclei of the brain of the codfish. *J. Comp. Neurol.* 17: 67-87.
- Hugelin, A. (1980). Does the respiratory rhythm originate from a reticular oscillator in the waking state? In: *The Reticular Formation Revisited*, ed. by J. A. Hobson and M. A. B. Brazier. New York, Raven Press.
- Iscove, S., J. L. Feldman and M. I. Cohen (1979). Properties of inspiratory termination by superior laryngeal and vagal stimulation. *Respir. Physiol.* 36: 353-366.
- Luiten, P. G. M. and J. N. C. van der Pers (1977). The connections of the trigeminal and facial motor nuclei in the carp as revealed by anterograde and retrograde transport of horseradish peroxidase. *J. Comp. Neurol.* 174: 575-590.
- Luiten, P. G. M. (1979). Proprioceptive reflex connections of head musculature and the mesencephalic trigeminal nucleus in the carp. *J. Comp. Neurol.* 183: 903-912.
- Poole, C. A. and G. H. Satchell (1979). Nociceptors in the gills of the dogfish, *Squalus acanthias*. *J. Comp. Physiol.* 130: 1-7.
- Satchell, G. H. (1959). Respiratory reflexes in the dogfish. *J. Exp. Biol.* 36: 62-71.
- Satchell, G. H. (1978). *Type J Receptors in the Gills of Fish*. Cambridge, Cambridge University Press, pp. 131-142.
- Shelton, G. (1959). The respiratory center in the tench (*Tinca tinca* L.). I: the effects of brain transection on respiration. *J. Exp. Biol.* 36: 191-202.
- St. John, W. M., R. L. Glasser and R. A. King (1972). Rhythmic respiration in awake, vagotomised cats with chronic pneumotaxic area lesions. *Respir. Physiol.* 15: 133-244.
- Sutterlin, A. M. and R. L. Saunders (1969). Proprioceptors in the gills of teleosts. *Can. J. Zool.* 47: 1209-1212.
- Trenchard, D. (1977). Role of pulmonary stretch receptors during breathing in rabbits, cats and dogs. *Respir. Physiol.* 29: 231-246.
- Trippenbach, T. and J. Milic-Emili (1977). Vagal contribution to the inspiratory 'off-switch' mechanism. *Fed. Proc.* 36: 2395-2399.